Antibacterial, anti-adherent and cytotoxic activities of surfactin(s) from a lipolytic strain Bacillus safensis F4

Faten Abdelli, Marwa Jardak, Jihene Elloumi, Didier Stien, Slim Cherif, Sami Mnif, Sami Aifa

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[1]Faten ABDELLI, [1]Marwa JARDAK, [1]Jihene ELLOUMI, [2]Didier STIEN, [3]Slim CHERIF, [1]Sami MNIF and [1*]Sami AIFA

[1]Laboratory of Molecular and Cellular Screening Processes, Centre of Biotechnology of Sfax, P.O. Box 1177, 3018

[2] UPMC UNIV PARIS 06, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Sorbonne Universities, Observatoire Oceanologique, 66650 Banyuls-Sur-Mer, FRANCE.

[3]Faculty of Sciences, Gafsa. University Campus Sidi Ahmed Zarrouk 2112 Gafsa. University of Gafsa.

*Corresponding author:

Prof. Sami Aifa

Centre of Biotechnology of Sfax (University of Sfax).

Sidi Mansour Road Km 6, BP 1177, 3018 Sfax, Tunisia

Email : sami.aifa@cbs.rnrt.tn

Phone: +216-74871816

Fax: +216-74875818

Abstract

The bacterial strain F4, isolated from olive oil-contaminated soil, has been found to produce biosurfactants as confirmed by oil displacement test and the emulsification index results. The identification of the strain F4, by 16S ribosomal RNA gene, showed a close similarity to *Bacillus safensis*, therefore the strain has been termed *Bacillus safensis* F4. The Thin Layer Chromatography (TLC) and the High Pressure LiquidChromatography-Mass Spectrometry (HPLC-MS/MS) demonstrated that the biosurfactant had a lipopeptide structure and was classified as surfactin. The present study showed also that the produced biosurfactant has an important antibacterial activity against several pathogen strains as monitored with minimum inhibitory concentration (MIC) micro-assays. In particular, it presented an interesting anti-planktonic activity with a MIC of 6.25 mg $mL^{-1}$and anti-adhesive activity which exceeded 80%against the biofilm-forming *Staphylococcus epidermidis* S61 strain. Moreover, the produced lipopeptide showed an antitumor activity against T47D breast cancer cells and B16F10 mouse melanoma cells with IC_{50} of 0.66 mg mL^{-1} and 1.17 mg mL^{-1}, respectively. Thus, our results demonstrated that *Bacillus safensis* F4 biosurfactant exhibited a polyvalent activity *via* a considerableantibiofilm and antitumoralpotencies.

Keywords:

Anti-adherent; anti-cancer; Biosurfactant; *Bacillussafensis* F4; Surfactin(s).

Introduction

Biosurfactants or bioemulsifiers are amphipathic surface-active molecules, which are produced by micro-organisms, composed of hydrophobic (nonpolar) and hydrophilic (polar) moieties. As aconsequence, theyhave the ability to aggregate at interfaces between fluids with different polarities such as oil/water or air/water, reducethe surface and interfacial tensions and form emulsions (Sen et al. 2017). These compounds are characterized as glycolipids, lipopeptides, lipopolysaccharides, fatty acids, phospholipids and neutral lipids (Bezza and Chirwa2016; Collaet al. 2010). Biosurfactantsare produced by a wide variety of bacteria, actinobacteria and fungi with different chemical structures. Some bacterial genera like *Bacill*us and *Arthrobacter*are known with their production oflipopeptidebiosurfactant (Sriram et al. 2011).Somestudies have described the biological activities of the biosurfactants including antimicrobial, anti-adhesive and anti-biofilm properties (Silva et al. 2014, Gudiña et al. 2010a).In fact, the bacterial infections and their biofilm formation abilities causing resistance increase against drugs is getting a serious problem for human health. An urgent need for solving this problem is based on the screening of novel drugs eradicating or inhibiting biofilm formation. The adherence is the first step of the infectious process that requires efficient antagonising molecules. Previous studies reported that based on their amphiphilic structures, the biosurfactants reduce the surface tension and therefore affecting the bacterial adherence (Janek et al. 2013).In this context, the lipopeptidebiosurfactant produced by *Bacillus subtilis* presented antibacterial, anti-adhesive and anti-biofilm activities on uropathogenic bacteria (Moryl et al.2015).Moreover, a glycolipid biosurfactant, presented cytotoxic activities on cancer cell lines,was produced by a *Nocardiafarcinica* strain(Christova et al. 2015). The biosurfactants, which are selective in nature, act on the surface of liquids and facilitate the action of certain enzymes such as lipases and/or esterasesby reducing the surface

tension of liquids and/or improving the solubility of water immiscible substrates (Sekhon et al. 2011, 2012).

Lipases are characterized by their ability to synthesize ester bonds in a non-aqueous media (Ülker and Karaoglu 2012) and their production can be associated with several factors including pH, temperature, carbon source and the presence of inducers such as oils and some biosurfactants (Cherif et al. 2011; Colla et al. 2010).

Nowadays, biosurfactants take an important scientific interest with their interesting proprieties such as the high biodegradability, lower toxicity, better environmental compatibility, and important specific activity at extreme conditions of temperature, pH and salinity (Sriram et al. 2011).

In this context, searching for novel biosurfactant producing strains with potential biosurfactant production is required. For that,lipolytic strains could be a possible original source of biosurfactant production (Sekhon et al. 2012).The present study describes the biosurfactant production by a lipolytic strain *B.safensis*F4 and investigatesits antibacterial, anti-adhesive and antitumor activities.

Materials and methods

Bacterial strains

B. safensis F4, *B. subtilis,Staphylococcus aureus*, *Enterococcus faecium,Micrococcus luteus,Agrobacterium tumefaciens, Salmonella enterica*, *Escherichia coli* and *Pseudomonas savastanoi*weregrown in LB (Luria-Bertani) medium.*S. epidermidis* S61, abiofilm-forming bacterium isolatedin our lab from the roof of an old house in Sfax, Tunisia(Jardak et al. 2017), was grown in Tryptic Soy Broth (TSB) medium.

Cell lines and cultures

Breast cancer T47D and mouse melanoma B16F10 cell lines, obtained from the American Type Culture Collection (ATCC), were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum, 50 IU/mL penicillin, 50 mg mL^{-1} streptomycin at 37°C in a humidified 5% CO_2 atmosphere.

Bacterial biosurfactantactivity

The oil displacement assay was performed according to Morikawa et al. (1993)using the Petri plate (90 mm diameter) filled with 25 mL of distilled water then 10 µL of a crude oil was added. 10 µL of a cell free culture supernatant was slowly placed on the center of the oil surface. The diameter of the clear halo zone was measured after 30 seconds of incubation.

The determination of the emulsification index (E24) is carried out according to the following equation(Cooper and Goldenberg 1987).E24 was measured using the cell free culture. Two millilitres of a vegetable oil were added to an equal volume of cell free supernatant and homogenized for 2 minutes at high speed. The height of emulsion layer was measured after 24h.All the experiments were done in triplicate.

E24 (%) = (Total height of the emulsified layer / Total height of the liquid layer) x100

Surface tension determination

Surface tension of the 24 h culture broth supernatant was measured according to the De Nouy methodology using a tensiometer TD1 (Lauda-K¨onigshofen, Germany). The measurement was performed in triplicate.

Identification of bacterial strain

Strain F4 was identified using the API 20E test *Enterobacteriacae*(BioMérieux, France) and by sequencing of the 16S rRNA gene. The genomic DNA of the strain F4 was extracted

following the protocol detailed by Wilson et al. (1987). The 16S ribosomal DNA of the strain F4 was amplified by PCR (Polymerase chain reaction) using the universal bacterial primers Fd1 and Rd1 (Fd1, 5'-AGAGTTTGATCCTGGCTCAG-3'; Rd1, 5'-AAGGAGG-TGATCCAGCC-3'), and the following program: denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec and extension at 72°C for 1 min 45 sec for a total of 30 cycles.

The PCR products were purified with a Favor Prep GEL/ PCR Purification Kit (FAVORGEN) and sequenced using the ABI PRISM, 3100.The obtained sequences were compared with other bacterial sequences in the NCBI database using BLAST program. The phylogenetic tree was constructed using the neighbour-joining method (Naruya and Nei 1987) by MEGA 4.0.

Bacterial biosurfactant production

*B.safensis*F4 strain was retained as the best local strain producing biosurfactant.The strain was incubated overnight at 30°C and 160 rpm in 250 mL shaking flasks with 100 mLof LB medium. Two millilitres of culture were used as inoculum and were cultivated in 500 mL shaking flasks containing 200 mL of the medium with 1% olive oil. The culture was incubated for 24 h at 180 rpm and 30°C to allow maximum biosurfactant production. Cell-free supernatant was obtained by centrifugation at 4°C during 20 min at 4000xg (ROTANTA 460 RF, Hettich). The obtained supernatant was treated by acidification to pH 2.0 using a3M HCl solution and incubated overnight at 4°C. Then, the acidified supernatant was extracted with ethyl acetate and concentrated with a rotary evaporator (Gargouri et al. 2016).

Thin layer chromatography (TLC)

The extracted biosurfactant in ethyl acetate was analysed by TLC. The sample dissolved in methanol was spotted on silica gel TLC plate (TLC Silica gel 60 F_{254}, Merck Darmstadt, Germany). The plate was developed with a mobile phase of chloroform/methanol/water

149 respectively in the ratio of 65:25:4 (v/v/v). The dried plate was sprayed with a solution of
150 0.25% ninhydrin in acetone and then, incubated at 105°C for 5 min (Janek et al. 2010).

151 **Biosurfactantpurification and identification**

152 The extracted biosurfactant in ethyl acetate was fractioned using solid-phase extraction (SPE)
153 (Alajlani et al. 2016). C_{18} Phenomenex strata-X column (silica gel, 10 g) was conditioned by
154 the elution of 3 volumes of acetonitrile. The sample was deposited on the surface of the silica
155 and drawn through the solvent. For the mobile phase, the HPLC (High Pressure Liquid
156 Chromatography) grade acetonitrile (100% - 3 volume column) was used in first step, then a
157 binary mixture of HPLC grade dichloromethane/ methanol (v/v - 3 volume column) was used.
158 The obtained eluates were collected and dried under vacuum. Finally, the acetonitrilefraction
159 was retained.

160 Two microliters of acetonitrile fraction diluted at 5 $mgmL^{-1}$ in methanol, were injected in a
161 Dionex Ultimate 3000 UHPLC-HESI HRMS Q-Exactive focus system (Thermo Scientific)
162 connected to Xcalibur software. The chromatographic separation was conducted followed the
163 protocol of Girard et al. (2017) with slight modifications. TheHypersil GOLD C_{18} column
164 (150 mm × 2.1 mm) with 1.9 µm particle size (Thermo Scientific) and constant flow rate of
165 0.5 mL min^{-1}. The column oven was set to 50°C. The water (eluent A) and acetonitrile (eluent
166 B) containing both 0.1% formic acid, were used as mobile phases. A gradient profile was
167 applied, starting with 5% of B and kept constant for 1 min. The percentage of B was linearly
168 increased to 100% in 15 min, and was kept at 100% for 9 min and returned to initial
169 conditions over 1 min. Four minutes of equilibration were followed, giving a total operating
170 time of 30 min. The instrument has been run in the full scan mode with a range of 100 to
171 1500m/z equipped with an electrospray interface (ESI). The polarity of the electrospray
172 interface was continuously switched between positive and negative polarity. The LB medium

was used as a control subjected to extraction with ethyl acetate. The common peaks between the chromatographs of the samples and the medium were not retained.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) for the lipopeptide, produced by *B.safensis* F4, was defined as the lowest concentration that inhibited the growth of microorganisms after 24 h. The test was performed against several human and plant pathogenicstrains (*S.aureus*, *E.faecium*, *M. luteus*, *A. tumefaciens*, *S. enterica*, *E. coli* and *P. savastanoi*) and *B. subtilis*. The choice of these strains is justified since we tried to maximize our chance for finding interesting molecules that could be applied to fight against human or plant bacterial infections. The biosurfactant anti-planktonic activity against *S. epidermidis* S61 was performed with the same test. Each bacterium was grown in LB medium overnight at 30°C. Bacterial cultures were then adjusted to an optical density of 0.6 at a wavelength of 600 nm.The crude biosurfactant was dissolved in Dimethylsulfoxide (DMSO) and then filtered. Serial dilutions were made to yield volumes of 100 µL per well with final concentrations ranging from 0.0125 to 25 mg mL^{-1}in LB medium. Twenty microliters of bacteriumovernight culture,with appropriate OD, wereadded to each well and a final volume of 200 µL per well was adjusted with medium. Wells containing just LB medium with inoculum and these containing medium, inoculum and Ampicillin served as controls. The plate was then incubated at 37°C for 24 h. Twenty microliters of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution at 1 mg mL^{-1} were added to each well.

The determination of the biosurfactant MICwas based on the MTT color change.In fact, the viable bacteria were detected by the change of yellow MTT color to purple. For that, the well devoid of bacterial growth(yellow color) was retained as MIC, which was expressed in mg mL^{-1}.The same test was carried out against Gram-positive and Gram-negative strains.

Anti-adhesive activity

The 96-well flat bottom plates were used for biofilm cultures (Mathur et al. 2006).

S. epidermidis S61, a biofilm-forming strain (Jardak et al. 2017), was grown overnight in TSB medium at 30°C and diluted with fresh medium supplemented with 2.25% (w/v) glucose.One hundred microliters of the bacterial culture dilution was added into each well to obtain a final $OD_{600\ nm}$ of 0.1. Then, 100 µL of *B.safensis*F4biosurfactant dissolved in TSB, containing 20% (v/v) ofDMSO, at various concentrations, were added into wells to reach final concentrations of 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 mg mL^{-1}. Wells containing onlyTSB medium supplemented with, 2.25% glucose and 20% (v/v) of DMSO, and bacterial suspension were served as controls.

Plates were incubated for 24 h at 30° C under static conditions. After incubation, the wells were emptied into a container by inverting the plates. Each well was gently washed twice with 250 µL of sterile phosphate buffered saline (PBS: 137 mMNaCl, 2.7 mM KCl;10 mM Na_2HPO_4 ; 1.76 mM KH_2PO_4; pH 7.2) in order to remove the planktonic cells (Beenken et al. 2003) . After washing, plates were dried at 60°C for 60 min. Then, wells were stained with 150 µL of crystal violet (0.2%) prepared in 20% ethanol for 15 min at room temperature(Vasudevanet al. 2003). After staining incubation, crystal violet was removed and excess dye was washed three times with sterile water. Finally, 200 µL of glacial acetic acid 33% was added to each well and plates were incubated for 1 hour at room temperature. The optical density (OD) was measured at 570 nm using a Varioskanmicroplate reader (Thermofisher).

The percentage of the adhesion inhibition wascalculated by the following formula:

[(OD (control*) - OD (treated strain)) / OD (control*)] x 100

*Control: untreated strain with the extract

The anti-adhesive activities of the crude biosurfactant and the acetonitrilefraction, against *S. epidermidis*S61 were confirmed by microscopic observations using the OLYMPUS fluorescent microscope BX50 equipped with a digital camera OLYMPUS DP70. The biofilms were grown on glass pieces (Ø 10mm) placed in 24-well polystyrene plates treated with the biosurfactant.Non-treated wells, containing TSB supplemented with 10% (v/v) of DMSO, served as controls (Padmavathi and Pandian 2014).The biosurfactant was added at a final concentration of 10 mg mL^{-1}in TSB with 10% (v/v) of DMSO. The bacterial inoculation was adjusted toan $OD_{600nm}$of 0.1. Plates were incubated at 30°C for 24 h. The wells were then carefully emptied with pipetting and glass slides were washed with sterile PBS (1X) before the treatment with 500 µL of acridine orange (0.1%, w/v, dissolved in PBS1X). Visualization was performed througha 40x objective using U-MWB2 filter with excitation at 460-490 nm and emission at 520 nm.

Cytotoxicity assays

T47D breast cancer and B16F10 mouse melanoma cells were grown in 96-well plates (Orange Scientific) until 40% confluence. Thebiosurfactant, wasadded at different concentrations (0.1, 1 and 10 mg mL^{-1}) and incubated for 48 hours at 37°C in a humidified atmosphere containing 5% CO_2.

The cell viability was assessed using the MTT assay as previously described by Mosmann (1983).After treatment, the medium was exchanged by a fresh one and 10 µL of MTT solution (5 mg mL^{-1}in PBS) were added. After incubation for 4 hours, 100 µL of 10% SDS (Sodium dodecyl sulfate) solution were added to each well to dissolve the formazan. The optical density was measured at 570 nm using a Varioskanmicroplate reader (Thermofisher). The growth inhibition was expressed according to the following formula:

$$\textbf{(\%) cell survival = (AT/A0) x100}$$

A0: control absorbance; AT: treated cells absorbance.

StatisticalAnalysis

All experimentsweredone in triplicate. The obtainedresults are expressed as mean values with the standard error. The statistical analyses wereperformedusingStudent's t-test to compare the controls and treatedsamples at a significancelevel of 5%.

Results and discussion

Selection of biosurfactant producing strain

Morphological and biochemical tests showed that the rod-shaped strain F4 was motile, Gram-positive, catalase-positive and oxidase-positive. Based on the phylogenetic analysis of the 16S rRNA gene sequences, the strain F4 was affiliated to the genus *Bacillus* with 99% of similarity to *Bacillus safensis* FO-36b^{T} (AF234854) (Fig. 1)and was termed as *B. safensis* F4.The 16S rRNA gene sequence, including 1378 nucleotides, was deposited in the GenBank nucleotide database under the accession number MF927780.

In fact, *B.safensis*F4 was retained after laboratory screening of lipolyticstrainsfor their ability to produce biosurfactant during growth on olive oil. The oil displacement assay showed that the selected strain presented the highest clear halo zone (about 21.08 ± 1.46 cm^2). The emulsification activity of the selected strain against sunflower oil was 74.99%. Previous results showed that *Bacillus cereus* NK1 biosurfactant presented a clear halo zone of 2.95 cm^2 and 62% in the oil displacement test and emulsification activity against *n*-hexadecane, respectively(Sriram et al. 2011).Ibrahim (2018) claimed that biosurfactant produced by *Ochrobactrumanthropi* HM-1 culture showed a clear halo zone 38.5 cm^2 , while 33.17 cm^2 was presented by *Citrobacterfreundii* HM-2 biosurfactant. The cell-free culture broths of

HM-1 and HM-2 strains successfully emulsified sunflower oil with approximately 70% and 60%, respectively.

Surface tension determination

Surface tension is a key parameter for the evaluation of biosurfactant production.In fact, a microorganism is considered as a promising biosurfactant producer, if it could reduce the surface tension to less than 40 mN m^{-1}(Shete et al., 2006). The obtained results showed that our biosurfactant is able to reduce surface tension until 30.73mN m^{-1}± 0.48 which is lower than results obtained by Ghazala et al.(2017) during the characterization of an anionic lipopeptide produced by *Bacillus mojavensis* I4 where the surface tension of the culture supernatant was 31.5 ± 0.8mN m^{-1}.Moreover, our results are very close to those obtained by Jemil et al., (2016) which showed that the best result in decreasing surface tension was observed with *Bacillus methylotrophicus*DCS1 strain (31 mN m^{-1}).Likewise, other study showed that biosurfactants produced by *O.anthropi* HM-1 and *C.freundii* HM-2 were able to reduce surface tension until 30.8 ± 0.6 and 32.5 ± 1.3 mNm^{-1}, respectively (Ibrahim 2018).While, compared to surface tensions of some chemical surfactants studied by Ghazala et al. (2017), *B.safensis*F4 cell free broth showed lower surface tension than SDS (34.8±1.3mN m^{-1}) and Triton X-100 (32±0.9mN m^{-1}).

Characterization of *B.safensis*F4 biosurfactant

TLC analysis showed that *B. safensis* F4 biosurfactant is a lipopeptide. Therelative front (R_f) value was 0.56(Fig. 2) which confirmed that the biosurfactant extract is a lipopeptide as reported by similar previous studies (Fernandes et al. 2007).

In order to identify our biosurfactant, the acetonitrile fraction was collected, and then analysedby LC-MS (Liquid chromatography–mass spectrometry) (Fig. 3). The details of the

obtained masses have been identified according to previous reported studies. Results showed the presence of two surfactinderivates ($M+H^+$=1022.6668 and 1008.6513/ M-H+=1020.6579 and 1006.6436, respectively) at retention time of 15.98 min with the presence of adducts (M+Na) (Table 2). The presence of surfactin was confirmed by the positive and negative ionizationmode (Jasim et al. 2016). At the same retention time of 15.98 min, the two compounds were identified as Leu/Ile-7, C_{14}surfactin and Leu/Ile-7, C_{13} surfactin with different masses of 1021.66 m/z and 1007.65 m/z (Price et al. 2007). Another peak at 9.20 min has been depicted ($M-H^+$ = 329.2328), which could correspond to pinellic acid. According to literature, pinellic acid is mainly known with its anti-allergic (Arulselvan et al. 2016) and anti-inflammatory (Nagai et al. 2004) activities.

Determination of minimum inhibitory concentration (MIC)

Das et al. (2008) reported that some types of biosurfactants produced by many *Bacillus*species present antimicrobial activity against many bacteria including pathogenic strains.

Our lipopeptide showed limited activity against Gram-negative bacteria compared to that obtained against the Gram-positive tested strains. The tested biosurfactant has a MICof 0.78 mg mL^{-1}against *B. subtilis*and 1.56 mg mL^{-1}against *S. aureus, E. faecium* and *M. luteus.* However, it presented a MIC value of 3.125 mg mL^{-1}against *A. tumefaciens, S. enterica, E. coli* and 1.56 mg mL^{-1}against *P.savastanoi*(Table 1).Moreover, Singh andCameotra (2004) reported that the lipopeptide produced by *B. subtilis* C1 was found to be active against several Gram-positive bacteria.

In another study, biosurfactant produced by the *Lactobacillus paracasei* ssp. *paracasei* A20 showed significant antimicrobial activities against pathogenic *E. coli, S.aureus* with MIChigher valuesranging between 25 and 50 mg mL^{-1}comparing with our results (Gudiña et al. 2010 b).Likewise, a high level of growth inhibition was observed against different

pathogens with a biosurfactant produced by *Lactobacillus helveticus*at a concentration of 25 mg mL^{-1}(Sharmaand Saharan 2016). Furthermore, many lipopetides produced by *Bacillus licheniformis,* (Yakimov et al. 2007; Fiechteer 1992) and *B. subtilis* (Vollenbroich et al. 1997) were known by their important antimicrobial activities. In other studies, the crude biosurfactant produced by *Lactobacillus jensenii* presented approximately 100% activity against *E. coli, and S. aureus* with a MIC of 50 mg mL^{-1}which are higher than our MICvalues (Sambanthamoorthy et al. 2014).

Concerning the anti-planktonic activity, the crude biosurfactant and the acetonitrile fraction were tested against *S. epidermidis* S61.Results showed that the crude biosurfactant and acetonitrile fraction effectively inhibited its growth with MIC of 12.5 mg $mL^{-1}$and 6.25 mg mL^{-1}, respectively. However, biosurfactantproduced by *L.helveticus* showed a high percentage of growth inhibition (98.4%) against *S. epidermidis* with a concentration of 25 mg mL^{-1} (Sharma and Saharan 2016).

Anti-adhesive activity

The ability of the crude biosurfactant and the acetonitrile fraction to inhibit the early biofilm formation at various concentrations was carried out against *S. epidermidis*S61. According to the Figure 4, the crude biosurfactant and the tested fractionsignificantly (P<0.001) inhibited the biofilm formation with approximately the same percentages of 90% and 80% at the concentrations of 10 and 5 mg mL^{-1},respectively. However, at the concentration of 2.5 mg mL^{-1}, the acetonitrile fraction, containing the surfactin, showedhigher anti-adherence activity with a percentage of inhibition of 64% against53% of the crude biosurfactant.Comparing with our results, the purified biosurfactant produced by *B. cereus* NK1 presented lower percentagesof biofilm inhibition of *S. epidermidis* at the raison of33.55% and 26.46% at concentrations of 10 and 5 mg mL^{-1}, respectively(Sriram et al. 2011).In similar studies,the

anti-adhesive activity of *B.methylotrophicus* DCS1 crudelipopeptide was evaluatedagainst different strains usingbiosurfactant pre-treated polystyrene surfaces. Results showed that the highest anti-adhesive effect was observed against *C. albicans* with an inhibition percentage of about 89.3% when biosurfactant was applied at a concentration of 1mg mL^{-1}(Jemil et al., 2017).In another study, the crude biosurfactant isolated from *L. paracasei ssp. paracasei* A20 inhibited the adherence of *S. epidermidis* at the concentration of 50 mg mL^{-1} with a percentage of 72.9% (Gudiña et al. 2010 b) compared to 90 and 80% at 10 and 5 mg mL^{-1} respectively of our present biosurfactant.

Moreover, biosurfactant produced by *L. helveticus* showeda potential anti adhesive activityagainst *S.epidermidis*with a percentage of 85%which is similar to our results but at higher concentration of 25 mg mL^{-1} (Sharma and Saharan 2016).Furthermore, biosurfactants produced by*L. jensenii* and *L. rhamnosus* presented anti-adhesive and anti-biofilm activities against the pathogen strains *A. baumannii, E. coli, and S. aureus* at concentrations ranging between 25 and 50 mg mL^{-1} (Sambanthamoorthy et al. 2014).

The anti-adherence activity of the two extracts was confirmed by fluorescence microscopy. The images of the acridine orange staining treated slides with extractsshowed the reduction in the biofilm covered surface compared to the control (Fig. 5). Lipopeptides are able to decrease biofilm surface and interfacial tension (Zhao et al. 2017). Previous studies demonstrated that biosurfactants had the ability to alter the surface characteristics of bacterial cells and reduce their adhesive properties. In fact, the application ofbiosurfactantto a substratum surface can decrease its hydrophobicity, interfere with the microbial adhesion and microorganisms adsorption process (Rodrigues et al. 2006a).

Cytotoxicity assays

363 The cytotoxicity assay of the crude biosurfactantand the acetonitrile fraction was performed
364 against T47D breast cancer cells and B16F10 mouse melanoma cells.
365 Figures 6 showed that the crude biosurfactant and the acetonitrile fraction showed high
366 inhibition against T47D and B16F10 cells at 10 mg mL^{-1}(P <0.001). Furthermore, at the
367 concentration of 1 mg mL^{-1}, the acetonitrile fraction was more toxic against B16F10 cells
368 with a survival of 59.75% than T47D cells,whereas, at concentration of the 0.1 mg mL^{-1}, both
369 tested samples did not show any toxicity against both cell lines.
370 The acetonitrile fraction inhibited significantly cancer cell growth at almost all the tested
371 concentrations (P <0.01).It presented an IC_{50} of 1.17 mg $mL^{-1}$and 0.66 mg mL^{-1}against
372 B16F10 cells and T47D cells, respectively (Fig. 7).
373 These results can be correlated with the composition of the acetonitrile fraction, which mainly
374 consists of surfactin, belonging to lipopeptides. According to literature, lipopeptides can act
375 as antitumor agents (Rodrigues et al. 2006b).
376 Previous studies reported that a biosurfactant extracted from *Lactobacillus casei*showed anti-
377 proliferative potencies against an epithelial cell line with an IC_{50} (The half-maximal inhibitory
378 concentration) ranging from 109.1±0.84 mg $mL^{-1}$to 129.7±0.52 mg mL^{-1} (Merghni et al.
379 2017)which are higher than the IC_{50} values obtained by our biosurfactant.
380 Moreover, it was previously demonstrated that surfactin could disrupt the membrane structure
381 via two main mechanisms which are insertion into lipid bilayers, modification of membrane
382 permeabilization via channel formation or diffusion of ions across the membrane barrier and
383 membrane solubilization by a detergent-like mechanism (Deleu et al. 2013, Wu et al.
384 2017).Interestingly, Gudiña et al. (2016) reported that the surfactin anticancer activity is in
385 relation with its hydrophobic nature. In fact, the fatty acid moiety of surfactin strongly
386 interacts with the acyl chain of the phospholipids in order to penetrate the outer sheet of lipid

bilayer, while the peptide moiety interacts with the polar head group of the lipids in cancer cells.

Conclusion

In the present study, the best producing biosurfactant strain has been screened and selected.Termed *B. safensis*F4, it is a lipolytic bacterial strain that has the propriety to produce surfactin with important surface-active properties. Crude and purified biosurfactant showed important antibacterial activity under planktonic conditions, preventing also bacterial adherence through inhibiting early stage biofilmformation. Interestingly, surfactin from *Bacillus* sp. F4 haspotent cytotoxic activity against cancer cell lines,T47D breast cancer cells and B16F10 mouse melanoma cells. These findings make this studied surfactin a good candidate for potential applications in preventing infectious diseases and treating cancer.

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Conflict of interest

The authors declare that they have no conflict of interest.

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